PATENT SPECIFICATION

NO DRAWINGS

858,784

Date of Application and filing Complete Specification: Aug. 26, 1958.
No. 27377/58.
Application made in Japan on Aug. 27, 1957.
Application made in Japan on Oct. 18, 1957.
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COMPLETE SPECIFICATION

Water-Soluble Methylhesperidins and their Production

CORRECTION OF CLERICAL ERROR/S

SPECIFICATION NO. 858,784

The following correction is in accordance with the Decision of the Superintending Examiner, acting for the Comptroller-General, dated the fourth day of May, 1961:-

Page 8, lines 23 and 27 and page 9, line 48, *for* "methyl glucosyl" *read* "methylglucosyl"

Page 8, line 30, *for* "dimethyl glucosyl" *read* "dimethylglucosyl"

Page 8, line 53, *for* "oxen" *read* "ox"

Page 10, line 62, *for* "hesperidine" *read* "hesperidin"

Attention is also directed to the following printer's errors:-

Page 3, line 3, *for* "C_{27} H_{36} O_{15}" *read* "C_{29} H_{36} O_{15}"

Page 3, line 11, *for* "C_{30} H_{39} O_{15}" *read* "C_{30} H_{38} O_{15}"

Page 3, line 14, *for* "disluphide" *read* "disulphide"

Page 3, line 24, *for* "C_{30} H_{39} O_{15}" *read* "C_{30} H_{38} O_{15}"

Page 11, line 4, *before* "an" *delete* "of"

Page 11, lines 18 and 21, *for* "hesperitin" *read* "hesperetin"

THE PATENT OFFICE,
12th June, 1961

DS 93401/1(5)/R.153 200 6/61 PL

The production of more effective water-soluble flavonoid derivatives having less toxicity has now been studied and success in producing several new compounds has been achieved. Further, the methylation of hesperidin with a view to obtaining more excellent and less toxic derivatives has been studied, and the chemical structures of the methylated products and their pharmacological properties, such as vitamin P-activity, and toxicities have been clarified.

[*Price 3s. 6d.*]

closed above can be obtained by methylation of hesperidin. But if hesperidin is methylated with such a methylating agent as dimethyl sulphate in an aqueous solution containing an alkaline agent, the product is a mixture of complicated composition including methylated hesperidin chalcone and several kinds of methylated hesperidin.

On the other hand, since the compounds which are excellent in medicinal effect and

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Water-Soluble Methylhesperidins and their Production

We, TAKEDA PHARMACEUTICAL INDUSTRIES, LTD., of 27, 2-chome, Doshomachi, Higashi-Ku, Osaka, Japan, a corporation under the Laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to water-soluble methylhesperidins and a process for producing them.

In 1936, L. B. Armentano and Szent-Györgyi et al. succeeded in extracting citrin from lemon juice and designated it as vitamin P (Deutsch. Med. Woschr. *62*, 1325 (1936)). Later it was clarified that the vitamin P effect of the substance is attributable to hesperidin which is the main principle of it. Since the effect of rutin was reported by J. Q. Griffith et al. (Proc. Soc. Expt. Biol. Med., *55* 228 (1944)), many investigations on substances having vitamin P-like effect and on bioflavonoids have been reported. C. W. Wilson has succeeded in obtaining methylated hesperidin chalcone through a path wherein hesperidin is first treated with concentrated alkali solution to produce its chalcone and the product is then methylated with dimethyl sulphate (United States Patent Specifications Nos. 2,425,291 and 2,612,015). The methylated hesperidin chalcone is stable over a wide pH range and its excellent medicinal effect was recognised by Bohr et al (J. Pharmacol. *92*, 243 (1949)). But this compound has a shortcoming in that its toxicity is relatively high.

The production of more effective water-soluble flavonoid derivatives having less toxicity has now been studied and success in producing several new compounds has been achieved. Further, the methylation of hesperidin with a view to obtaining more excellent and less toxic derivatives has been studied, and the chemical structures of the methylated products and their pharmacological properties, such as vitamin P-activity, and toxicities have been clarified.

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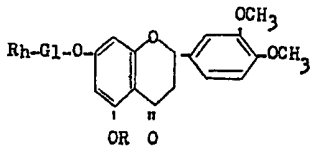
Thus a new group of compounds which are fundamentally different from the known methylated hesperidin chalcone, and which have far weaker toxicity and stronger medicinal effect, has now been formed.

The water-soluble methyl hesperidins included in the present invention belong to a group of compounds represented by the general formula shown below:



wherein R is a hydrogen atom or a methyl radical, Rh is rhamnose or methylrhamnose and Gl is glucose or methylglucose.

In the above general formula, the methylrhamnose and/or methylglucose may have a methyl radical in any position and the number of such methyl radicals is not limited.

Among the water-soluble methylhesperidins of the present invention are included, for example, 3^1 - methyl - 7 - (2(?) - methylrhamnosyl - 2 - methylglucosyl) - hesperetin, 3^1 - methyl - 7 - (rhamnosyl - 2 - methylglucosyl) - hesperetin and 3^1 - methyl - hesperidin.

Each of these water-soluble methylhesperidins has stronger vitamin P-activity, less toxicity and higher solubility in water when compared with known methylated hesperidin chalcones.

The water-soluble methylhesperidins disclosed above can be obtained by methylation of hesperidin. But if hesperidin is methylated with such a methylating agent as dimethyl sulphate in an aqueous solution containing an alkaline agent, the product is a mixture of complicated composition including methylated hesperidin chalcone and several kinds of methylated hesperidin.

On the other hand, since the compounds which are excellent in medicinal effect and

have low toxicity are methylated hesperidins, it is desired to find a method which produces only methyl derivatives of hesperidin and inhibits the formation of derivatives of hesperidin chalcone as completely as possible. After strenuous investigation success has been achieved in producing water-soluble methylhesperidin as the main product of the methylation of hesperidin, in reducing the formation of methylated hesperidin chalcone, and in separating the chalcone compound impurity from the desired compounds.

The invention thus includes a method for producing water-soluble methylhesperidin, which comprises methylating hesperidin under conditions wherein the formation of methylated hesperidin chalcone is inhibited, and then separating effective components of low toxicity from the reaction mixture.

The following Table shows that water-soluble methylhesperidins have excellent medicinal effects and lower toxicities as compared with methylated hesperidin chalcones. In the Table, "Medicinal effect" means "prolongation of dye appearance time in the irritated area" in experiments using rabbits as the test animal and Evans blue as the dye. The test is conducted on the basis of the principle reported by A. M. Ambrose and F. DeEd in J. Pharmacol. *90*, 359 (1947), and on the basis of the fact that the activity to correct permeability of capillary vessels, one of the vitamin P-effects, can be determined by measuring the prolongation of the time necessary for leaking dye out of the vessels. The value of the prolongation is expressed as a percentage increase of the time necessary for initial leakage of dye in the treated group as compared with the time in a control group. The toxicities of the compounds are shown in the Table as LD_{50} (intravenous) of mice in mg. per Kg. of body weight.

TABLE

No.	Compound	Medicinal effect (%)	Toxicity LD_{50} (mg/kg)
1	3¹-Methyl-7-(rhamnosyl-2-methyl-glucosyl)-hesperetin	116.7	750
2	3¹-Methyl-7-(2(?)-methylrhamnosyl-2-methyl-glucosyl)-hesperetin	32.9	900
3	3¹-Methylhesperidin	38.6	800
4	Water-soluble methylhesperidins obtained by the method of this invention - mixture of methyl-hesperidins, including the above three compounds	95.8	850
5	3,6¹-Dimethylhesperidin chalcone	19.8	150
6	3,6¹-Dimethyl-4¹-(rhamnosyl-2-methyl-glucosyl)-hesperetin chalcone	19.4	450

The detailed physico-chemical properties of the above compounds are itemised below:—

In the following description, temperatures are all uncorrected and Rf-values were measured by the following method: a sample is subjected to paper-chromatography by the ascending method using a solvent-system of ethyl acetate.benzene.ethyl alcohol.water (86:14:2:50) and magnesium acetate - ethyl alcohol reagent is sprayed on the chromatogram, which is then irradiated with ultraviolet light.

1. 3^1-Methylhesperidin

Colourless needles: M. Pt. 149—150° C. Rf = 0.18

Molecular formula: $C_{27}H_{36}O_{15}$

Solubility: Soluble in water. Slightly soluble in methanol, ethanol, acetone and ethyl acetate. Insoluble in ether, petroleum ether, benzene, chloroform and carbon disulphide.

Positive to $FeCl_3$-reaction and HCl-Mg-reaction (Ethanolic solution). Ultraviolet absorption of this compound resembles that of hesperidin.

2. 3^1-Methyl-7-(rhamnosyl-2-methylglucosyl)-hesperetin

Fine colourless needles: M. Pt. circa 80° C. Rf = 0.45

Molecular formula: $C_{30}H_{39}O_{15}$

Solubility: Soluble in water, methanol, ethanol, acetone, ethyl acetate and chloroform. Insoluble in ether, petroleum ether, benzene and carbon disluphide.

Positive to $FeCl_3$-reaction and HCl-Mg-reaction.

Ultraviolet absorption spectrum of this compound resembles that of hesperidin.

3. 3^1-Methyl-7-(2(?)-methylrhamnosyl-2-methylglucosyl)-hesperetin

Colourless powdery crystals: M. Pt. circa 80° C. Rf = 0.82

Molecular formula: $C_{31}H_{40}O_{15}$

Soluble in hot ether and hot benzene.

Other properties resemble those of the above two compounds.

4. 3^1,5-Dimethylhesperidin

Colourless needles: M. Pt. 145—146° C.

Molecular formula: $C_{30}H_{38}O_{15}$

Solubility and other properties resemble those of 3^1-methyl-herperidin above described.

5. Water soluble methylhesperidins obtained by the method of this invention—a mixture of methylhesperidins including the above four compounds.

Content of methoxyl groups (—OCH_3): 15.5%
Solubility: Very readily soluble in water (1:1).

Soluble in methanol and ethanol. Slightly soluble in isopropyl alcohol, chloroform and ethyl acetate. Insoluble in ether, benzene and petroleum ether.

$FeCl_3$-reaction: Positive (dark brown)

HCl-Mg-reaction: Positive (yellowish red)

6. 3,6¹-Dimethylhesperidin chalcone

Rf = 0.08

7. 3,6¹-Dimethyl-4¹-(rhamnosyl-2-methylglucosyl)-hesperetin chalcone

Rf = 0.35

In this Specification we use the expression "water-soluble methylhesperidin" to mean one of various kinds of methylated derivatives of hesperidin when the term is used in the singular, and to mean a mixture of two or more of such compounds when it is used in the plural.

In the process of this invention, hesperidin is methylated under such conditions that the formation of methylated hesperidin chalcones is inhibited. In general, flavanone derivatives such as hesperidin are reversibly changed into their chalcone, for instance under strongly alkaline conditions as noted in Wilson's United States Specifications, supra. When hesperidin is methylated with such a methylating agent as dimethyl sulphate in the presence of an alkaline reagent at a temperature over about 30°C., it is said that methylated derivatives of hesperidin chalcone are chiefly produced. On the other hand, if the reaction is conducted at nearly room temperature, the reaction gives a mixture of methylated derivatives both of hesperidin and of hesperidin chalcone. It has now been found that the methylating temperature required for inhibiting the formation of chalcone compounds is a temperature below room temperature, especially below 10°C. However, when the alkaline agent is calcium hydroxide, the formation of the chalcone derivatives is strongly inhibited. Therefore, in such cases the temperature of the methylation is not necessarily required to be restricted.

The methylation process of this invention may be effected by a number of methylating agents, such as dimethyl sulphate, methyl halides and diazomethane, in the presence of an alkaline agent such as a hydroxide or carbonate of an alkali metal or alkaline earth metal. It has been found that the amount of alkaline agent used in the methylation may be not more than 5 moles to 1 mole of hesperidin. Such amount of alkaline agent is enough to inhibit the formation of chalcone derivatives. When the alkaline agent is used in an amount of over 5 moles to 1 mole of hesperidin, methylated hesperidin chalcone is formed as a by-product and when over 10 moles of the alkaline agent is used, methylated hesperidin chalcone is the chief product. On the other hand, the yield of water-soluble methylhesperidins may be lowered when the amount of the alkaline agent is less than 2 moles to 1 mole of hesperidin. However, when the hydroxide of an alkaline earth metal such as calcium hydroxide is used as the alkaline agent, water-soluble methylhesperidin may chiefly be produced when over 10 moles of the alkaline agent is used, in other words in such a case the formation of the chalcone derivatives is well inhibited. However, even when hesperidin chalcone is once formed by the alkalinity of the solution, it can be reconverted into hesperidin when the pH value of the solution is lowered by the addition of such a reagent as a halide of an alkaline earth metal so that the hydroxide of the alkaline earth metal is formed in the reaction mixture. The methylation of hesperidin may thus be effected as smoothly as when the reaction is conducted utilising only the hydroxide of the alkaline earth metal as the alkaline reagent.

To inhibit the formation of the chalcone derivatives, it is most preferable that 1 mole

of a methylating agent be used to 1 mole of hesperidin. The formation of the chalcone derivatives is increased when a greater proportion of the methylating agent is used.

The methylation process of this invention may be carried out in a suitable solvent. Water or an inert solvent miscible with water, such as acetone and dioxane, may be used as the solvent. Use of an organic solvent promotes the reaction, and use of such an organic solvent as ether and benzene may inhibit the decomposition of hesperidin by alkali, i.e. the formation of chalcone derivatives.

The above-disclosed effects of the proportion of methylating agent and the reaction temperature are illustrated in the Tables given below:

Relationship between the amount of methylating agent and the composition of the product (methylating agent: Dimethyl sulphate)

Methylating Agent (mole)	Water-soluble methylhesperidin (%)*	Methylated herperidin chalcone (%)*
1	62.3	37.7
2	50.8	49.2
2.2	44.4	55.6
4	43.9	56.1
5	32.5	67.5

* % shows relationship between their total methyl groups.

Influence of reaction temperature on the composition of the product (methylating agent: Dimethyl sulphate)

Temperature (°C.)	Amount of methylating agent to 1 mole of hesperidin (mole)	Water-soluble methyl-hesperidin (%)*	Methylated hesperidin chalcone (%)*
3 — 5	2.2	49.2	50.8
	4	62.7	37.3
	5	61.1	38.9
15 — 20	2.2	44.4	55.6
	4	43.9	56.1
	5	32.5	67.5

* (%) is as above.

As the reaction mixture contains various impurities besides several different water - soluble methylhesperidins and a small amount of methylated hesperidin chalcone, they have to be removed from the mixture. To remove such impurities from the reaction mixture, such methods as salting out, dialysis, extraction with an organic solvent, absorption, elution from an adsorbent, separation with an ion-exchanger and distribution between two solvents, may be used. These steps may be conducted singly or jointly, once or repeatedly. For example, an aqueous solution of the crude product of this invention containing the chalcone derivatives is saturated with a water-soluble inorganic salt such as sodium chloride, whereupon the water - soluble methylhesperidins separate out almost completely, most of the by-products remaining in the aqueous

solution.

Alternatively, impurities such as water-soluble inorganic compounds can be removed by dialysis against flowing water.

On the other hand, an aqueous solution of the impure water-soluble methylhesperidins may be shaken with an organic solvent which is not freely miscible with water, such as *n* - butanol, *iso* - butanol, *sec.*- or *tert.* - butanol or methyl ethyl ketone, to leave only impurities in the aqueous layer. In this case, the pH of the aqueous solution is preferably adjusted to about 4 to 5. Alternatively, the aqueous solution may be saturated with a water-soluble inorganic salt, such as common salt, when the water-soluble methylhesperidins transfer completely into the organic solvent layer. Water-soluble impurities contained in the organic solvent layer may be removed by washing the layer with an approximately 15% aqueous solution of common salt.

As the water-soluble methylhesperidins are liable to be adsorbed in various adsorbents, a solution of the water - soluble methylhesperidins may be brought into contact with a suitable adsorbent to purify the product. As the adsorbent there may, for example, be used alumina, magnesium silicate, magnesium oxide, diatomaceous earth, activated charcoal or an ion - exchanger. To bring the aqueous solution of the water-soluble methylhesperidin into contact with the adsorbent, there are two different procedures which may be adopted (i) the solution is made to flow through a tower packed with an adsorbent, or (ii) a mixture of the solution and an adsorbent is agitated. Of the two processes, the former is better for industrial purposes because the manipulation is easier than the latter. The water - soluble methylhesperidin thus adsorbed may be eluted with a suitable solvent. The solvent may be selected from those which can readily dissolve the compounds in question, for example acetic esters such as ethyl acetate and butyl acetate, alcohols such as methanol, ethanol and butanol, and ketones such as acetone and dioxane. A mixture of such solvents, with or without water, may be employed for the purpose. The elution may be effected smoothly by adding benzene, chloroform, or the like to the solvent. The most preferable solvent for elution is selected experimentally. By such a step, impurities such as inorganic salts may be removed.

Even the above treatment cannot remove the undesired yellow-coloured chalcone derivatives completely, and therefore the product has slight yellowish colour. It has, however, been found that methylated hesperidin chalcone can be completely removed from the product by treating the aqueous solution of the impure product with an alkali metal or ammonium bisulphite (HSO_3—) or a reagent which is changeable into such a salt in an aqueous solution, such as pyrosulphite (S_2O_5—) or hyposulphite (S_2O_4—). For this purpose, such a reagent is added to an aqueous solution of the impure product. A similar effect can also be obtained by the following method: the crude product is dissolved in an aqueous solution of alkali bicarbonate and sulphur dioxide is passed through the solution to form an alkali bisulphite in the reaction system. The water - soluble methylhesperidins free from chalcone derivatives may be separated from the reaction mixture thus obtained. In this reaction, it is believed that the methylated hesperidin chalcone forms an addition product with a bisulphite, and that water-soluble methylhesperidins are hardly affected. The addition product is readily soluble in water and hardly soluble in organic solvents in general. Though the reaction conditions may be selected in accordance with the composition of the material, the amount of the reagent may generally be over 2 moles to each mole of methylated hesperidin chalcone contained in the material. Most preferably 5 to 10 moles of the reagent may be used. If other conditions are suitable, greater amounts of the reagent may be employed. The reaction may generally be conducted at pH 5—7, and at a temperature not higher than 80°C. Whatever preferable conditions other than temperature may be selected, the yield of water-soluble methylhesperidin is lowered, or undesirable sulphur-containing by-products may be produced, if the reaction temperature is over 80°C. The reaction is generally completed within 2 to 3 hours, and the end-point of the reaction can easily be noted from the disappearance of the yellowish colour from the solution. If the pH - value and/or temperature is unsuitable, the complete purification of the product cannot be attained—for example, (1) if the reaction is conducted at pH 4.4, the addition product is not produced even when the reaction is continued for about three weeks, and (2) if the reaction is conducted at 90°C. the purity of the water-soluble methylhesperidin remains at about 60—70%.

For extracting the purified water-soluble methylhesperidin, the reaction mixture is shaken with an organic solvent. As the organic solvent *n* - butanol may suitably be used, but a solvent which is not freely miscible with water such as *iso* - butanol, *sec.*- or *tert.* - butanol or methyl ethyl ketone, may also be employed. To recover the water-soluble methylhesperidins from the extract, the solvent is distilled off, preferably under reduced pressure, and the residue is recrystallised from a suitable solvent such as ethanol or isopropanol.

The above-explained process for separating methylated hesperidin chalcones as their bisulphite-addition product from water-soluble methylhesperidin is a peculiar method applicable only to the products of this invention. Of

course, the removal of the methylated hesperidin chalcone may be effected by conventional means such as chromatography, which are used for separating two or more compounds whose natures and/or structures are analogous. But they are unsuitable for industrial purposes because their manipulation is always complicated and difficult.

As has already been mentioned the crude product of the present invention is a mixture comprising several kinds of water-soluble methylhesperidins. Therefore, the components can, if necessary, be separated from each other, and the separation may be effected simply by, for instance, adsorption chromatography.

Chromatography can effect a series of operations such as removing inorganic impurities, separating chalcone derivatives and isolating each desired component.

For effecting the chromatographic separation, a solution containing one or more of the water-soluble methylhesperidins, methylated hesperidin chalcones and organic or inorganic impurities is prepared, and the solvent may, for instance, be acetone or an acetic ester. The solution is poured into a column packed with an adsorbent, and the components are developed with a suitable developing solvent, in accordance with the conventional liquid chromatography, to separate them from each other.

When an ion-exchanger is used as the adsorbent, an aqueous solution of the material is passed through a column packed with a cation exchanger, and the compounds in question may then be eluted separately from the column with a suitable solvent such as diluted ethanol, preferably 30—50% ethanol. The cation exchanger may be a weakly acid form such as a methacrylic acid type exchange resin (for example that sold by Rohm & Haas Co. under the trade name Amberlite IR—50, "Amberlite" being a Registered Trade Mark).

In this case, the inorganic ions contained in the material are preferably removed in advance by the method previously explained because the existence of inorganic ions disturbs the functioning of the ion-exchanger.

When the column partition chromatography is conducted utilising the difference in distribution-coefficient of the components in two solvents, an adsorbent such silica gel, filter paper powder or starch may be used as the carrier. Development may be conducted according to the conventional method using water saturated with a suitable organic solvent as the stationary phase, and a suitable organic solvent saturated with water as the mobile phase. Thus the components are developed on the carrier, forming their bands according to their Rf-values, and then each component is successively eluted out.

On a similar principle to that of the aforementioned column partition chromatography, the separation of each component may conveniently be conducted by the counter-current distribution process, that is to say the material is dissolved in a stationary phase similar to the above, and distribution and extraction are conducted continuously between the stationary and mobile phases.

According to the above-mentioned processes each component is obtained as a solution. From the solution, the solvent is distilled off under reduced pressure at a low temperature to obtain a crude product. Each of the crude components may be purified by recrystallisation from a suitable solvent, such as an alcohol, and preferably from isopropyl alcohol. Thus each water-soluble methylhesperidin can be obtained separately.

Before the separation of each water-soluble methylhesperidin is conducted by the above method, it is convenient to conduct a preexamination into the kind and content of each component contained in the material by by paper partition chromatography. For example, a butanol extract of the reaction mixture is subjected to paper partition chromatography by the ascending method using ethyl acetate.benzene.ethanol.water (86:14:2:50), and the chromatogram is sprayed with an alcoholic solution of magnesium acetate and then irradiated with ultra-violet light. If the sample is obtained by the reaction of 1 mole of hesperidin, 2 moles of sodium hydroxide (10% aqueous solution) and 2 moles of dimethyl sulphate, a blue-coloured fluorescence can be observed at Rf-values of 0.18, 0.45 and 0.82, each of which is the spot of a water-soluble methyl-hesperidin, and the admixed methylated hesperidin chalcones give yellowish spots at Rf-values of 0.08 and 0.35, under visible light.

Each component contained in the reaction mixture has the previously explained chemical structure and medicinal effects. They have vitamin P-like activities in living things including human beings, and may be administered to human beings orally or by injection. However, a mixture of water-soluble methylhesperidins containing hardly any chalcone derivatives may also be used for the same purposes, and the mixture is easier to use by reason that its production is simple as disclosed above, that its toxicity is less than that of the known methylated chalcone derivatives, and that the solubility of the components in water is high.

The following Examples illustrate and explain the actual working of the present invention, but do not set any limitations upon the scope of this invention. The temperatures given in the Examples are all uncorrected.

EXAMPLE 1

To a solution of 6.1 g. of hesperidin (0.01 mol) in 25 cc. of 8% sodium hydroxide solution (0.05 mol) is added dropwise 6.3 g. of dimethyl sulphate (0.05 mol) with stirring and cooling to below 10°C. After standing

over-night, the reaction mixture is adjusted to pH 5 and filtered, and the filtrate is saturated with sodium chloride, whereupon a resinous substance separates out. The resinous substance is dissolved in 20 cc. of distilled water and salted out again with sodium chloride. The substance is then dried under reduced pressure at a temperature below 60°C. and the residue is dissolved in 30 cc. of isopropyl alcohol. After decolorising with 1 g. of activated charcoal, the solution is cooled with ice, whereupon a crystalline substance separates out.

The product is washed several times with 20 cc.-portions of ether to obtain a yellowish white crystalline powder melting at about 95°C. The yield is 4.5 g.

The production is a mixture of

3^1 - methyl - 7 - (rhamnosyl - 2 - methylglucosyl) - hesperetin

3^1 - methyl - hesperidin and

3^1 - methyl - 7 - (2 - (?) - methyl-rhamnosyl - 2 - methyl glucosyl) - hesperetin

together with

3^1 - 6^1 - dimethyl - 4^1 - (rhamnosyl - 2 - methyl glucosyl) - hesperetin chalcone and

2^1 - 3 - 6^1 - dimethyl - 4^1 - (rhamnosyl - 2,3 - dimethyl glucosyl) - hesperetin chalcone

as side products.

EXAMPLE 2

To a solution of 6.1 g. of hesperidin in 100 cc. of 50% aqueous dioxane are added dropwise 25 cc. of an 8% aqueous solution of sodium hydroxide and 6.3 g. of dimethyl sulphate alternately with stirring and cooling to below 15°C. The mixture is allowed to stand overnight, adjusted to pH 5 and concentrated to dryness under reduced pressure at a low temperature, and the residue is dissolved in 50 cc. of water. The solution is decolorised with 1 g. of activated charcoal and sodium chloride is added, whereupon a resinous substance separates out. The same treatment of the substance as used in Example 1 gives a yellowish white crystalline powder. The yield is 4.1 g. The powder is the same as in Example 1.

EXAMPLE 3

The filtrate of a reaction mixture obtained as in Example 1 is dialysed in an oxen intestinal membrane bag against flowing water for 15 hours and decolorised with 1 g. of activated charcoal. The solution is then concentrated to dryness under reduced pressure at a low temperature and treated as in Example 1. The yield is 3.8 g. of the same product as in Example 1.

EXAMPLE 4

The filtrate of a reaction mixture obtained as in Example 1 is shaken four times with 20 cc.-portions of *n* - butanol and the combined extracts are washed with 10 cc. of 15% aqueous solution of sodium chloride and dried over 20 g. of calcium chloride. The solution thus obtained is concentrated to dryness under reduced pressure at a low temperature and processed as in Example 1. The yield is 4.2 g. of the same product as in Example 1.

EXAMPLE 5

The filtrate of a reaction mixture obtained as in Example 1 is passed through a glass tube diameter, 7.5 cm.; length 30 cm.; packed with 100 g. of magnesium silicate and the column is washed with distilled water to remove sodium monomethyl sulphate. Then 200 cc. of 20% methanol is passed through the column to elute the product, and the eluate is decolorised with 1 g. of activated charcoal, filtered, and concentrated to dryness under reduced pressure at a low temperature. Further treatment of the residue is effected as in Example 1. The yield is 4.0 g. of the same product as in Example 1.

EXAMPLE 6

To the filtrate of a reaction mixture obtained as in Example 1 is added 50 cc. of isopropyl alcohol, and the mixture is adjusted to pH 6.5—7.0 and filtered. The filtrate is concentrated to dryness under reduced pressure and the residue is dissolved in 200 cc. of distilled water. The solution is passed through two towers successively, one of them being packed with 100 cc. of a polymethacrylate anion exchange resin and the other with a polystyrene cation exchange resin (the flow rate is 0.1 respectively, i.e. the volume of effluent eluted per hour was 1/10th of the apparent volume of the exchange resin in the chromatographic column), and the effluent is decolorised with 1 g. of activated charcoal and filtered. The filtrate is concentrated to dryness under reduced pressure at a low temperature and the residue is treated as in Example 1. The product is the same as in Example 1.

EXAMPLE 7

A solution of 6.1 g. of hesperidin in 200 cc. of methanol is boiled with 15 g. of methyl iodide and 3 g. of potassium carbonate on a water-bath for 3 hours. After cooling the reaction mixture, undesirable inorganic compounds are removed by filtration and the filtrate is concentrated to dryness. Recrystallisation of the residue from absolute ethanol gives 4.5 g. of yellowish powder. This powder comprises 3^1 - methyl - hesperidin and 3,6^1 - dimethylhesperidin chalcone.

EXAMPLE 8

To a suspension of 6.1 g. of hesperidin in 200 cc. of methanol is added an ethereal solution of diazomethane produced from 30 g. of nitrosomethylurea, when the reaction proceeds slowly, evolving N_2 gas. After the reaction is continued for 48 hours with shaking, the unreacted hesperidin (3.5 g.) is separated by filtration and the filtrate is concentrated to dryness. Recrystallisation of the residue from

isopropanol gives 2 g. of pale yellow powder, which contains 3^1 - methylhesperidin as the main component and small amounts of 3^1,5 - dimethylhesperidin and 3,6^1 - dimethylhesperidin chalcone.

EXAMPLE 9

To the milk of lime prepared from 1.8 g. of calcium hydroxide and 50 cc. of water is added 6.1 g. of hesperidin, and then 6.3 g. of dimethylsulphate is added dropwise with stirring. The mixture is yellow at first but gradually becomes clear orange. The reaction is completed after 3 hours and the pH at that time is 7. The same treatment of the reaction mixture as is used in Example 1 gives 4.5 g. of the same product as in Example 1.

EXAMPLE 10

To the milk of lime prepared from 1.4 g. of calcium oxide and 50 cc. of water is added 6.1 g. of hesperidin and the mixture is treated in the same way as in Example 9.

EXAMPLE 11

To a solution of 6.1 g. of hesperidin in 25 cc. of 8% sodium hydroxide solution is added a solution of 2.75 g. of calcium chloride in a little amount of water and the mixture is then treated as in Example 9.

EXAMPLE 12

A solution of 10 g. of a crude methylated product (content of water-soluble methylhesperidins, 65%) and 1.7 g. of acidic sodium sulphite in 75 cc. of water is kept at 70°C. for 2 hours. The solution is bright yellow at first but is gradually decolorised as the reaction proceeds. Ten grams of sodium chloride are added to the reaction mixture, whereupon a white resinous substance is salted out. Recrystallisation of the substance from isopropanol gives 5.8 g. of colourless crystalline powder, the purity of which is 98.6% (by spectrometric examination). Methylhesperidin chalcones are not contained in the product which consists of

3^1 - methyl - 7 - (rhamnosyl - 2 - methylglycosyl) - hesperetin

3^1 - methyl - hesperidin and

3^1 - methyl - 7 - (2(?) - methylrhamnosyl - 2 - methyl glucosyl) - hesperetin

EXAMPLE 13

A solution of 20 g. of a crude methylated product (content of water-soluble methylhesperidins, 93%) and 3.3 g. of sodium pyrosulphide in 80 cc. of water is adjusted to pH 6.0 with sodium bicarbonate and kept standing for 2 hours at 40°C. The reaction mixture is extracted with four 50 cc.-portions of *n* - butanol and the combined extracts are concentrated to dryness under reduced pressure. Recrystallisation of the residue from isopropyl alcohol gives 15 g. of colourless crystalline powder. The purity is 98.5%. The product is the same as in Example 12.

EXAMPLE 14.

A solution of 10 g. of a crude methylated product (content of water-soluble methylhesperidins, 90%) and 1.5 g. of sodium hyposulphite in 40 cc. of water is kept standing for 6 hours at room temperature. The same treatment of the reaction mixture as in Example 12 gives water-soluble methylhesperidin, the purity of which is higher than 95%. Methylhesperidin chalcones are not contained in the product.

EXAMPLE 15

A suspension of 150 g. of magnesium silicate in 500 cc. of acetone is poured into a glass tube (7.5 cm. in diameter (30 cm. in length), and after several hours the acetone is drawn off from the bottom of the tube. The column is washed with an additional 100 cc. of acetone to prepare a column for chromatography. A clear solution of 5 g. of methylated hesperidin derivatives in 50 cc. of acetone (a little amount of water may be added, if necessary) is poured on the column and the components are developed with a solvent system of ethyl acetate.benzene.ethanol, water (86:14:2:50). The rate of outflow of the solvent is about 0.5—1.0 cc. per minute, and the effluent is collected in fractions of 10 cc. each. The fractions positive to HCl - Mg reaction are grouped according to their Rf-value on a paper chromatogram and the fractions having the same Rf-value are combined. Each of the combined fractions is concentrated under reduced pressure, whereupon the respective component separates out.

In this way, 3^1 - methyl - 7 - (2(?) - methylrhamnosyl - 2 - methyl - glucosyl) - hesperetin, 3^1 - methyl - 7 - (rhamnosyl - 2 - methylglucosyl) - hesperetin and 3^1 - methylhesperidin are obtained from the 14th to 23rd fractions, the 24th to 40th fractions, and the 90th to 127th fractions, respectively. The minimum respective yields are 150, 600 and 1500 mg. over a number of similar experiments.

The remaining fractions also contain a minute amount of the active components, which can be collected by the same chromatography as above.

EXAMPLE 16

A glass tube packed with 100 g. of powdered filter paper is washed with a solvent system of ethyl acetate.benzene.ethanol.water (86:14:2:50) and left standing overnight, filled with 200 cc. of the solvent to make the solvent permeate into the column homogeneously. A solution of crude methylated hesperidin derivatives in a little amount of the same solvent is poured on the column and the components are developed with the same solvent. The effluent is collected in fractions of 10 cc. each and the desired compounds are recovered as in Example 15.

EXAMPLE 17

A portion of 500 mg. of crude methylated hesperidins is shaken with a solvent system of ethyl acetate.benzene.ethanol.water (86:14:2:50) for 5 minutes in an apparatus

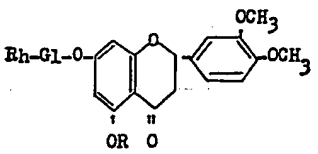
for the counter-current distribution method having 30 shaking tubes and left standing for 5 minutes. The shaking and standing are repeated alternately tc extract the components. Each of the fractions 2nd to 5th, 9th to 18th and 21st to 24th is concentrated under reduced pressure to obtain 3^1 - methylhesperidin, 3^1 - methyl - 7 - (rhamnosyl - 2 - methylglucosyl) - hesperetin and 3^1 - methyl - 7 - (2(?) - methylrhamnosyl - 2 - methylglucosyl) - hesperetin, respectively. The minimum respective yields are 100, 100 and 50 mg. over a number of similar experiments.

EXAMPLE 18

A glass tube (1.5 cm. in diameter, 100 cm. in length) packed with 100 cc. of a polymethacrylate anion exchange resin is treated with N—NaOH and N—HCl. A solution of 1 g. of methylated hesperidins is poured on the column at the flow rate of 0.05 to 0.1. The column is washed with 100 cc. of distilled water and the water-soluble methylhesperidins are eluted with 30% methanol fractionally. The effluent is collected in fractions and the desired compounds are recovered separately as in the preceding Example.

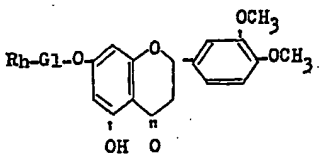
WHAT WE CLAIM IS:—

1. A compound having the formula



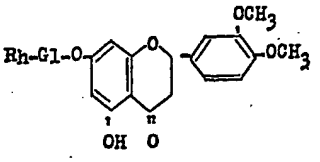
wherein R is a hydrogen atom or a methyl radical, Rh is rhamnose or methylrhamnose and Gl is glucose or methylglucose.

2. A compound having the formula



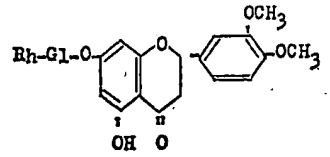
wherein Rh is rhamnose or methylrhamnose and Gl is glucose or methylglucose.

3. A compound having the formula



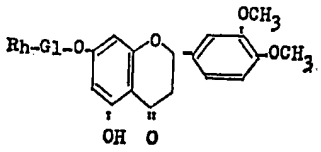
(wherein Rh is 2(?) - methylrhamnose and Gl is 2 - methylglucose) and having a melting point of about 80°C. and a Rf-value, as hereinbefore defined, of 0.82.

4. A compound having the formula



wherein Rh is rhamnose and Gl is 2 - methylglucose.

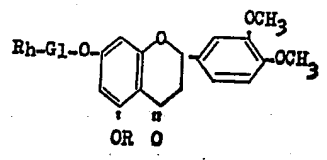
5. A compound having the formula



wherein Rh is rhamnose and Gl is glucose.

6. A mixture composed of compounds having the general formula represented in Claim 1.

7. A process for producing water-soluble methylhesperidins represented by the formula



wherein R is a hydrogen atom or a methyl group, Rh is rhamnose or methylrhamnose and Gl is glucose or methylglucose, which comprises methylating hesperidin under such conditions that the formation of methylated hesperidine chalcone is substantially inhibited, and removing the chalcone admixed with the reaction product.

8. A process as claimed in claim 7, wherein hesperidin is reacted with a methylating agent in an aqueous solution of not more than 5 moles of an alkaline agent per mole of hesperidin.

9. A process as claimed in claim 8, wherein the alkaline agent is sodium hydroxide and the reaction proceeds at a temperature below 10°C.

10. A process as claimed in claim 8, wherein the alkaline agent is calcium hydroxide.

11. A process as claimed in any of claims 8 to 10, wherein the methylating agent is dimethyl sulphate, methyl halide or diazomethane.

12. A process as claimed in any of claims 8 to 11, wherein the removal of the chalcone type impurities is effected by the following steps: the water-soluble methylhesperidins

which contain the chalcone type impurities are dissolved in water; the solution is adjusted to pH 5 to 7, an aqueous solution of a bisulphite, pyrosulphite or hyposulphite of of an alkali metal or ammonium is added, and the impurities are removed by salting out or extraction with an organic solvent.

13. Processes for producing compounds as claimed in any of claims 1 to 6, substantially as hereinbefore described.

14. Compounds as claimed in any of claims 1 to 6, whenever prepared by a process as claimed in any of claims 7 to 13.

15. A process for the separation of any two or more of the undermentioned compounds from each other:

(1) 3^1 - methyl - 7 - (rhamnosyl - 2 - methylglucosyl) - hesperitin,

(2) 3^1 - methyl - 7 - (2(?) - methylrhamnosyl - 2 - methylglucosyl) - hesperitin of melting point about 80°C. and Rf-value, as hereinbefore defined, of 0.82,

(3) 3^1 - methylhesperidin, and

(4) 3^1,5 - dimethylhesperidin,

which comprises subjecting a mixture composed of any two or more of the above compounds and other impurities to a column chromatography.

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